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EXPERIMENTS WITH A FILTRABLE VIRUS IN A TRANSFERABLE ILLNESS OF CANARY BIRDS

(Following is the translation of an article by W. Kikuth and H. Gollub, Hygienic Institute of Duesseldorf, Academy of Medicine and Chemo Therapeutic Institute of I.G. Farben Co., Elberfeld, which appeared in the German language periodical Zentr. Bact. Paras., 125, pages 313-20 (1932). Translation performed by Constance L. Lust.)

In chemotherapeutic trials with fowl malaria in canaries Kikuth observed the sudden increase in virulence of the Proteosoma strains. These had showed no variation in virulence during many passages over several years.

Canaries injected with P. praecox usually show a 20% lethality, but this rose sharply and finally reached 100%. The infection was much accuter and the malaria parasites were more numerous in the blood than previously.

It soon became apparent that there was actually no increase in virulence of the Proteosoma strains, but the virulence was increased by another, unknown factor.

If birds ill with malaria were treated with atabrine or plasmobrine from the day of blood inoculation, the malaria infection could be cured, but the birds still died on day 7-12 and no parasites were found in the blood.

Our first thought was that a mixed infection due to bacteria was responsible, which had been transmitted via the blood. It was previously observed that canaries have a high mortality. Wasielewski pointed out that canaries often die of coccidiosis. Marks discusses lethal infection generally in the bird. Edwards and Beaudette reported on a epizootic bacterial infection and Fourneau had to stop his chemtherapeutic experiments with canaries because of contamination. Sternberg cultured from canary heart, spleen and liver a gram-negative bacterium which agglutinated paratyphus-B-serum.

In this case coccidiosis was easily ruled out. Bacterial growth experiments on various media were completely negative. Therefore a very small virus had to be suspected as a causative factor. Filtration work confirmed these thoughts. They became the basis for this report. But, since this problem is rather complex, and many other questions were raised that remain unanswered, this report must be viewed as preliminary.

If canaries are injected intramuscularly with blood from ill birds, the bird showed no illness during the first few days. From day 4-8 the birds became papthetic, sit still and eat much less. Three-four days prior to death they look very sick. The feathers are touseled and the head lays to one side, and gasps for air. A liquid exudes from their bill. They die soon thereafter. No paralysis was seen and the stomach and intestines function normally.

Temperature remains normal, 42-43°. When the animals are very ill it drops below 42° and reaches 37-38° prior to death. All infected animals dis between day 7-12.

The breast muscle at the injection site behaves characteristically. After 4-5 days it turns yellow-brown and becomed edematos.

Red and white cells in blood show no alterations. Hemoglobin remains at 60-70%. Just before death young polychromatic erythrocytes increase.

In several birds we noted peculiar forms in the blood. These remain unexplained. They were not found in all birds. Interestingly these were also found in stock birds which appeared ill, even though they were, of course, not infected with malaria. We succeeded in culturing and passing viruses from these birds. We did this four times from our stock colony of birds.

Morphologically this was an irregular form found external to the red cell. In a Giemsa stained streak one sees unstained vacuoles. In the vacuoles one sees red-stained masses which appear granular. We wanted to know what this noteworthy formation represents and from where they originate. We were convinced that this was not related to protozoa. They were unregular and without structure. They did not move in an unstained preparation. We thought at first it may be degenerated polychromatic erythrocytes. However, in birds ill with malaria or anemic birds we found no such forms. The malaria ill birds have elevated numbers of polychromatic erythrocytes. At this time we cannot say more about this relationship.

We sent our preparation to Dr. Bayon-Cambridge and he believes the forms represent disease-altered blood platelets. It is known that thrombocytes of birds, contrary to mammals, contain nuclei. The question arises; whether blood platelets of birds are similar to those of mammals. Bird platelets, even though much smaller, have a certain similarity with erythrocytes; they are oval-round with a central nucleus. The cytoplasm is darkly colored (polychromatophilic tone).

According to Bayon the forms are reaction vacuoles in the cytoplasm of altered thrombocytes. A thorough study of blood preparations does not fully support this. We now think they arise due to some influence that the platelets have on the surrounding membranes of the thrombocyte. The nucleus may fall out of this structure causing the formation of a vacuole. Interestingly the forms are slightly bigger than platelets.

Further support for this hypothesis comes from the fact that in birds that have large numbers of forms have only very few normal platelets. We studied clotting time (suggested by Bayon) in sick birds. If the process involves the thrombocytes one would expect clotting time to be much slower. This was exactly true. Normal birds = 30-50 seconds (43 average): ill birds 1-3 minutes (average 1 minute 45 seconds).

Only one bird (of many) of the control, stock group had a time of 2 minutes. But, this bird died several days later and we could grow out the virus on other birds after IM injection of the heart blood.

Clotting time is normal in experimentally infected birds during the first days. Only prior to death when the bird is definitely ill does it increase.

All the facts assembled point to platelet which is "activated" by the virus (appearance of forms in infected birds and in control birds with virus culturable etc.). The question is not yet completely resolved.

Birds that die at the high point of illness after transfer via IM injection seldom have altered pictures in their inner organs; sometimes spleen enlarged, lungs lymph nodes secondarily enlarged. All other organs no change. The peritonium and pleura may have hemorrlogic lesions as described in flow pest.

Only the brest muscle is changed at the inoculation site. This is characteristic and appears visually 3-4 days prior to death. The color becomes noticably more yellow-brown. It is necrotic and gelatineous in nature. Microscopically interstitial large, uni-nucleated cells with vacuoles and lipid bodies are found. Many muscle fibers are necrotic. No evidence for bacterial infection. Skin, however, shows some ulceration.

We used infectious blood diluted with saline to inject IM to 0.3 ml saline 3-4 drops of venous blood. The injections gave very repeatable results. Birds died at 7-12 days after inoculation; 9 days average. We have grown out the virus 2 times in passages; one to 31 passages; once to 70 passages and still growing.

The virus remained infectious for three months without loss of virulence. The viremic blood was diluted 10^{-2} with steril H_2O and stored in sealed vials in the cold room. After 2-3 months birds were infected IM.

We diluted virus with distilled water and injected IM 0.3 ml into birds. Dilution of 1: 10,000 and 1:1,000,000 were infectious; $1:10^7$ was not infectious. The infection ran its course the same for both dilutions and this in turn was identical to the usual blood inoculation. Birds in the two dilutions died in 10-13 days. Therefore the illness duration was longer. The birds that were injected a 10^{-7} dilution and controls were successfully infected after 6 weeks with infected blood. So, no immunity was produced here.

We could not localize the virus further in blood; both serum and RBC were infective.

The virus is not stable to heat. One hour at 60°C destroys all activity of infection.

We filtered the virus; 0.1 ml blood and 10 ml H_2O was filtered in Berkefeld-N-candles under a pressure of 40-60 mm. Coli and staph controls were negative. Seitz filters did not work (no infectivity).

Experiments with altenuated virus - for production of immunity have been wholly negative so far. We are working on this aspect. We have seen no immunity since all birds die of the infection.

We believe that this virus is often a course of epidemics in canary birds. It is possible that this virus was responsible in Fourneau and Sternberg's work and that their bacterial infection was only a secondary phenomenon.

Recently a similar disease was observed in the Hamburg Tropic Institute, whereby all animals died only after an increase in virulence occurred. From Professor Giemsa we received two infected birds, which had been injected with blood of ill animals. We succeeded by further passages in identifying this disease as the same one we had. Birds died after 12 days with all characteristic signs. Giemsa could also find no bacterial basis for his illness.

Similar epidemics occur in song birds in nature. Maggiora and Valenti reported about an outbreak near Modena. They clearly demonstrated a virus. They could transfer it to starlings, eagle and owl, not to sparrows or pigeons. It may be related to our virus.

We were interested in the way that the infection comes about under natural conditions. Direct contact transmission may appear as likely even though no data are available. Other parasites may transfer the agent. Preliminary work is in progress about this.

The virus may be transferred parenterally and orally. We put emulsions of virus infected organs into the bird food. This infected the birds and virus could be isolated from blood. In the oral transmission the duration of illness is much longer. In one trial up to 52 days. The blood from this bird infected birds which died in 9-10 days with the usual clinical symptoms present.

If birds eat infected blood the same general picture follows. After a time the animal died and virus was present in their blood.

It is apparent that if the virus is not transferred IM it can remain in the organism, even in blood, for very long times without being able to demonstrate virus. It may be that via this route only very few organisms get into the organism. This is maybe true for the following

experiment. Blood was taken daily from the wing vein and transferred to other canaries. The virulence of the virus rises as the infection progresses, so that animals die due to the infection earlier than usual.

Blood of day after inoculation	Death of injected bird
day 3	after 12 days
" 4	" 10 "
" 5	" 9 "
" 6	" 9 "
" 7	" 7 "
" 8	" 6 "

The illness time can be reduced by 50% if the blood is taken from infected birds shortly before death. The picture of the disease from oral transmission which is characterized by a long infectious course, is similar to spontaneous illnesses from which the virus has been cultured. We could culture the virus from our stock colony of birds (birds come from several sources). In the first passage it is hard to say whether it is virus or another infection; therefore several passages are done. Birds injected IM in the first passage also only die at 20-27 days. Only in the second etc. passage does the infection run as described with the characteristic signs. No other changes occur then.

We tried to transfer the virus to pigeons and chicks. Particular interest was for chicks because a certain degree of similarity of fowl pest existed for our virus. These studies were completely negative. We injected IM 7 pigeons, 2 chicks, 2 young chickens and 6 roosters. The animals all remained well with no signs of illness.

We did finally succeed in transferring the virus to sparrows. The bought sparrows were observed for a long period and then injected IM with infected blood. All five birds died in 10-16 days with similar clinical signs as seen for canaries. The virus could be retransferred to canaries from sparrow blood.

The virus was transferred to finches (Orizornis orizivora). This can occur spontaneously and leads to a latent infection. These birds may then be carriers which is of interest epidemiologically. We will report more later on this.

From a differential diagnostic view only the results of Maggiora and Valenti are important. We could illiminate fowl pest. It does have many features in common; ie, pathological picture of breast muscles. The clinical course is somewhat different, especially duration. Our canaries were ill by day 4-5; dead by day 7-12. Their virus was more virulent in starlings; death occurred at day 1-3. It may be a varient of the virus, or it may be identical. Comparative studies must await further work.

Summary

A filterable virus (Berkefeld-N-Candle filter) is described, which occurred spontaneously in canaries and may cause epidemics. It is highly pathogenic killing animals 100% in 7-12 days. Unusual forms occurred in blood of infected birds and appear to be related to infection. They may arise from altered, degenerated blood elements. The virus is still infectious at 10^{-6} dilutions; stable for 3 months in cold, but labile to 60°C.

IM transmission causes necrotic, degenerative alterations at the site of injection. It cannot be transferred to pigeons, chicks, chickens, but can be to sparrows. Virus may be latent in certain birds (finches) which might be classified as virus carriers.